Changes of sperm quality parameters in Caspian roach (*Rutilus rutilus caspicus*) during spawning migration

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ABSTRACT: In this study, changes of pH, ionic (Na^+, K^+, Ca^{2+}, and Mg^{2+}), biochemical (total protein, glucose, and cholesterol) compositions of seminal plasma, sperm motility traits (percentage of motile spermatozoa and sperm movement duration), and sperm production characteristics (sperm volume, spermatocrit, and sperm density) were studied in Caspian roach, *Rutilus rutilus caspicus*, during spawning migration. Sperm of 10 males was collected three times during the spawning migration (in February, March, and April). The results showed that sperm motility parameters (percentage of motile spermatozoa and sperm movement duration) changed significantly ($P < 0.05$) during the reproductive season, but sperm density, spermatocrit, and sperm volume did not show significant differences during spawning migration. Analyses performed at each sampling time (February, March, and April) showed significant differences ($P < 0.05$) in calcium, magnesium, potassium, and cholesterol, whereas there were no significant changes in Na^+, pH, total protein, glucose, and cholesterol.

Keywords: spawning season; spermatological parameters; biochemical parameters; semen; roach

The Caspian roach (*Rutilus rutilus caspicus*) belonging to the family of Cyprinidae has been considered in fishery in the southern part of the Caspian Sea (Coad, 1980; Abdoli, 2000). Due to good taste and culinary customs of local people there has been a great demand for this fish. The population of the Caspian roach has been decreased due to overfishing and deterioration of its spawning ground and it is therefore considered a threatened species (Kiabi et al., 1999). The roach is a migratory fish which enters the border river Atrak and other Iranian rivers (e.g. the Gharesoo and the Gorganrood) for spawning. The reproduction migration in the southern Caspian Sea starts from January–February and continues until April. The fish travel about 70–80 km upriver (Petr, 1987). Most migration takes place at water temperature of 10–12°C. Gamete quality in species with annual spawning cycles varies during the spawning season (Buyukhatipoglu and Holtz, 1984; Piironen, 1985; Koldras et al., 1996; Rideout et al., 2004). Spermatogenesis in fish is regulated by endogenous (reproductive endocrine system) and environmental (thermoperiod or photoperiod) stimuli (Crim, 1982). The physiological changes that occur after transfer are controlled by endocrinological system, which regulates spermatogenesis and spermiation (Billard et al., 1995). In freshwater species, matured spermatozoa need a hypo-osmotic shock for triggering initiation of sperm motility (Morisawa et al., 1983). The duration of sperm motility is usually very short (a few minutes in freshwater species) and could be influenced by external environmental factors such as pH, temperature, ions, and osmolality (Alavi and Cosson, 2006). Seminal plasma characteristics (such as osmolality, pH, ionic, and biochemical compounds) and Adenosine triphosphate (ATP) content of sperm are known as parameters that influence sperm motility (Alavi and Cosson,

2006). In cyprinids, because of high osmolality, spermatozoa are immotile in the seminal plasma (Morisawa et al., 1983). Also, osmolality of seminal plasma differs significantly during reproductive season (Alavi et al., 2008a). The parameters like seminal plasma composition, spermatozoa concentration, spermatocrit, and sperm motility traits have been used for evaluating the quality of sperm (Alavi and Cosson, 2006). Assessment of sperm quality based on these parameters provides us with applied approaches to improve methods for artificial reproduction by developing immobilizing or activating media for fertilization (Rodina et al., 2004). Studying the effects of these factors on sperm quality can help establish good activation and/or immobilizing media for improving either artificial fertilization or cryopreservation protocols. It has been already shown the sperm quality parameters change during the spawning season in carp (*Cyprinus carpio*), tilapia (*Oreochromis mossambicus*) (Kruger et al., 1984), rainbow trout (*Onchorhynchus mykiss*) (Munkittrick and Moccia, 1987), and common barbel (*Barbus barbus*) (Alavi et al., 2008a). The changes could be in terms of spermatozoa concentration, sperm volume, seminal plasma composition, pH or sperm motility parameters such as percentage of motile spermatozoa and sperm velocity (Billard et al., 1995; Alavi et al., 2008a). Sperm characteristics also differ among species especially in terms of seminal plasma composition (Ciereszko, 2008) or sperm motility (Alavi et al., 2008a). These differences should be considered for developing artificial fertilization or sperm cryopreservation (Billard et al., 1995; Linhart et al., 2003). In this study, we determined pH, ionic (K^+, Na^+, Ca^{2+}, and Mg^{2+}), biochemical (total protein, glucose, and cholesterol) composition of the seminal plasma, sperm production characteristics (spermatocrit, sperm density, and sperm volume), and sperm motility parameters (sperm movement duration and percentage of motile spermatozoa) during spawning migration in the Caspian roach.

MATERIAL AND METHODS

Brood fish were caught in the Gorganrood River (36°44' to 37°49'N latitude and 54°42' to 56°28'E longitude) three times during the spawning season – at its beginning (February 10th), in the middle (March 3rd), and at the end (April 5th). Sperm of 10 mature males was collected by pressing gentile of the abdomen at each sampling time (male mean weight ± SE: 52.57 ± 9.8 g, 56.78 ± 11.4 g, and 51.12 ± 6.5 g at each sampling time respectively). Attention was taken to avoid contamination of sperm with blood, urine, and faeces. Sperm was kept on ice and immediately transported to the laboratory for analyses. According to procedure of Bozkurt et al. (2008) sperm motility was triggered directly in activation medium of 0.3% NaCl at ratio 1 : 1000 and immediately recorded with a 3 CCD video camera Panasonic WV-CP240 (Panasonic Corp., Osaka, Japan) mounted on a dark-field microscope (Leica Camera, Allendale, USA). The duration of sperm motility was measured immediately after initiation of sperm activation until 100% of the spermatozoa were immotile. The percentage of motility was defined as the percentage of progressively motile spermatozoa within each activated sample. Progressively motile spermatozoa were defined as actively swimming in a forward motion. Only forward moving sperm was judged motile and sperm cells that vibrated at the place were considered motile. Observations were made within 2 h of semen collection. The spermatocrit was defined as the ratio of volume of white packed material to the total volume of semen × 100 (Rurangwa et al., 2004). Microhaematocrit capillary tubes (75 mm in length, 1.1–1.2 mm in diameter) were filled with semen and the end of each tube was sealed with clay. The capillary tubes were centrifuged at 3000 *g* for 8 min (Sigma, Washington, USA). Measurements were taken in triplicate for each sample, and the average of the three measurements was used for the results. Spermatozoa density was calculated using the haemocytometer method. With this aim, semen was diluted with a saline solution (0.07 NaCl) (Alavi et al., 2009) and then a droplet (1 µl) of diluted semen was placed on a Thomas haemocytometer slide (depth 0.1 mm) with a coverslip and counted using light microscopy. After a few minutes (to allow sperm sedimentation), the number of spermatozoa was counted at magnification 200× and expressed as spermatozoa × 10^9/ml. Sperm volume was measured in graduated tubes and expressed in ml. All experiments were performed in triplicate at room temperature (20–22°C). To analyze the chemical components of seminal fluid, the semen was centrifuged (Eppendorf AG, Hamburg, Germany) at 4000 *g* for 8 min and then seminal plasma was collected. Plasma was centrifuged twice to avoid

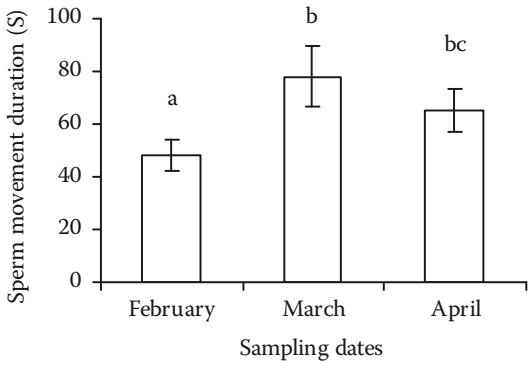

Figure 1. Changes in sperm movement duration of Caspian roach during spawning period. Different superscripts indicate means that were significantly different ($P < 0.05$, $n = 30$ males)

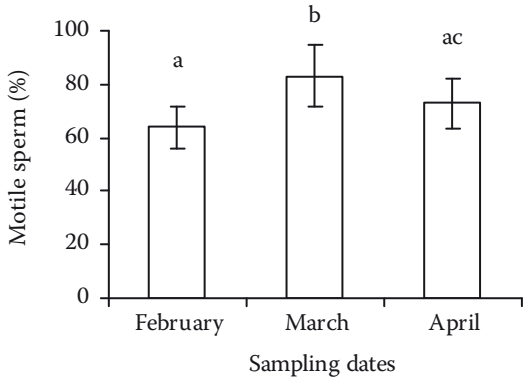

Figure 2. Changes in percentage of motile spermatozoa of Caspian roach during spawning period. Different superscripts indicate means that were significantly different ($P < 0.05$, $n = 30$ males)

possible contamination with spermatozoa. The seminal plasma pH was determined immediately before freezing using a Micro pH meter 762 (Saba Co., Tehran, Iran) and then samples were frozen at –20°C until analysis. Two minerals (Ca^{2+} and Mg^{2+}) and three biochemical parameters (total protein, glucose, and cholesterol) of the seminal plasma were measured using the spectrophotometer S2000-UV/VIS (Ocean Optic Company, Edinburgh, UK). Potassium and sodium were determined with a flamephotometer Jenway PFP7C (Triad Scientific Co., New Jersey, USA) (Standard kits from Parsazmoon Co., Tehran, Iran). Statistical data were analyzed using SPSS software (Version 11.5, 1996). One-way analysis of variance (ANOVA) was carried out to determine variation on sperm movement duration, percentage of motile spermatozoa, sperm density, spermatocrit, semen volume, seminal plasma pH, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, total protein, glucose, and cholesterol during spawning season. Before data analysis by ANOVA, Duncan test was used for normality of data distribution and homogeneity of variance. The Duncan test was used for comparison between means at a 0.05 significance level. All values were expressed as mean ± standard error (SE).

RESULTS

The spermatological parameters of the semen are presented in Figures 1–5. Sperm movement duration and percentage of motile spermatozoa increased from the beginning to the middle of the spawning period, towards the end of the reproductive season the values decreased ($P < 0.05$; Figures 1 and 2). On the other hand, no significant differences were observed in sperm density,

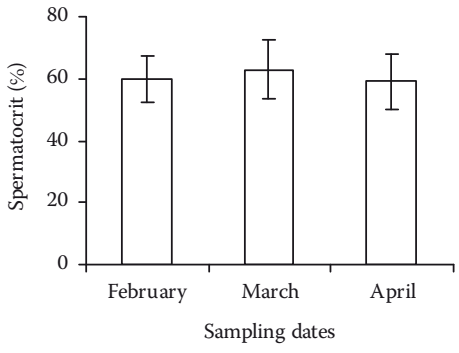

Figure 3. Spermatocrit of Caspian roach at different sampling times during the spawning period ($n = 30$ males)

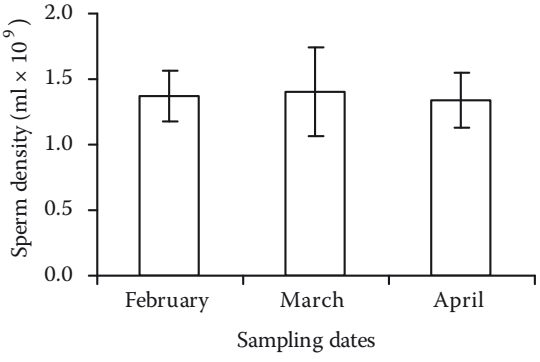

Figure 4. Sperm density of Caspian roach at different sampling times during the spawning season ($n = 30$ males)

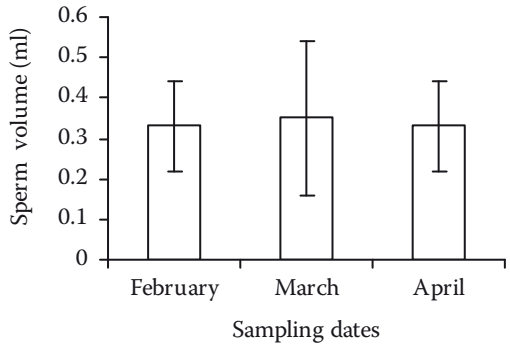

Figure 5. Sperm volume of Caspian roach at different sampling times during the spawning season (n = 30 males)

spermatocrit, and sperm volume during spawning migration (Figures 3–5). As shown in Table 1, higher values of Ca^{2+}, Mg^{2+}, and cholesterol were recorded in the middle of the spawning period compared to its beginning or the end. K^+ ion concentration decreased gradually from the beginning of the spawning period towards its middle and terminal part. The pH of seminal plasma did not show significant change during spawning season. No significant changes were observed in terms of the seminal plasma organic composition (total protein and glucose) during spawning migration.

DISCUSSION

Fish spermatozoa are different in the initiation (Cosson et al., 1995), duration (Billard and Cosson, 1992), and pattern of motility (Boitano and Omoto, 1992) among species. Changes in the quality of semen during spawning season have been reported in teleost fish (Billard et al., 1977). In this study, significant changes were observed in the percentage of motile spermatozoa and sperm movement duration, results are similar to those reported in rainbow trout (*Onchorhynchus mykiss*), brook trout (*Salvilenus alpinus*), and Atlantic salmon (*Salmo salar*) (Benau and Terner, 1980). No significant changes were reported in percentage of motile spermatozoa of Barbus (*Barbus barbus*) during the reproductive season (March–May) (Alavi et al., 2008a). Decreases in sperm motility have been documented in several fish species such as rainbow trout (Munkittrick and Moccia, 1987), turbot (Suquet et al., 1998), sea bass (*Dicentrarchus labrax*) (Dreanno et al., 1999), haddock (*Melanogrammus aeglefinus*) (Rideout et al., 2004), Atlantic halibut (*Hippoglossus hippoglossus*) (Babiak et al., 2006), cod (*Gadus morhua*) (Rouxel et al., 2008), and perch (*Perca fluviatilis*) (Alavi et al., 2010) as the reproductive season progressed. Decrease in percentage of sperm motility might be related to the osmolality of semen (Alavi et al., 2010). Several studies have revealed that decrease in sperm motility is due to ATP content which is necessary for sperm motility (Perchec et al., 1995). Nevertheless, more studies are needed to find correlations between ATP content and sperm motility parameters (Alavi et al., 2009). Lahnsteiner et al. (2004) demonstrated that sperm motility pattern changed during spawning season. Differing results on changes of sperm motility over the reproductive season may be due to changing endocrine performance involved in sperm maturation (Rideout et al., 2004; Alavi et al., 2008b). Sperm motility and sperm density are often used to estimate sperm quality (Krol et al., 2006). Seasonal variation of sperm density depends on fish species – a decrease was observed in sea bass (Dreanno et al., 1999; Fauvel et al., 1999)

Table 1. Changes in compositions of the seminal plasma of thirty male Caspian roach during the reproductive season

Parameter	February 10th	March 3rd	April 5th	Statistics
Na^+ (mM/ml)	200.05 ± 2.10	200.01 ± 3.01	200.70 ± 3.01	ns
K^+ (mM/ml)	34.40 ± 4.80^a	23.96 ± 1.50^b	23.73 ± 3.30^c	$P < 0.05$
Ca^{2+} (mM/ml)	10.29 ± 0.56^C	14.13 ± 0.70^a	12.27 ± 0.34^b	$P < 0.05$
Mg^{2+} (mM/ml)	3.96 ± 0.46^b	4.05 ± 0.38^a	2.47 ± 0.25^C	$P < 0.05$
Protein (mg/dl)	2.48 ± 0.48	2.67 ± 0.79	3.30 ± 0.19	ns
Glucose (mg/dl)	4.30 ± 0.71	4.95 ± 0.12	4.95 ± 0.45	ns
Cholesterol (mg/dl)	27.60 ± 0.39^b	62.45 ± 2.37^a	25.21 ± 0.71^b	ns
pH	7.45 ± 0.07	7.60 ± 0.14	7.95 ± 0.07	ns

$^{a–c, C}$different letters correspond to significantly different results

ns = nonsignificant

while an increase was recorded in Atlantic salmon (Piironen, 1985), turbot (Suquet et al., 1998), and Atlantic halibut (Babiak et al., 2006). There was no significant change in sperm density of roach during spawning migration. A decrease in sperm density towards the end of spermiation period has been reported in many teleosts such as tench (Zuromska, 1981), rainbow trout (Buyukhatipoglu and Holtz, 1984; Munkittrick and Moccia, 1987), turbot (Suquet et al., 1998), seabass (Dreanno et al., 1999), cod (Rouxel et al., 2008), and *Barbus barbus* (Alavi et al., 2008b). The mechanism regulating semen hydration during spermiation plays a major role in determining the sperm volume in fish (Alavi et al., 2008a, b). It has already been claimed that the sperm volume changes from the beginning to the end of spermiation (reproductive season). In this study no significant change was observed during spawning migration for sperm volume. In landlocked salmon (*Salmo salar m. sebago* Girard), sperm volume increased during the reproductive season (Piironen, 1985). Endocrinological events that regulate spermiation and milt hydration make the comparisons difficult (Mylonas et al., 1997; Vermeirssen et al., 2004). Also Campos-Mendoza et al. (2004) noted that variation in day length during spawning period affected the sperm volume in males. Seminal plasma not only maintains immobility of spermatozoa but also protects them (Cosson et al., 1997); this is the case, for example, in the common carp *Cyprinus carpio* (Toth et al., 1995) and in the rainbow trout *Oncorhynchus mykiss* (Billard, 1986). The ionic composition may change during the spawning season (Alavi and Cosson, 2006). Significant changes were observed in ionic composition (K^+, Ca^{2+}, and Mg^{2+}) of seminal plasma in this study. Similar results have shown significant variation in seminal plasma composition at different times of the reproductive season in common carp (Koldras et al., 1996), landlocked salmon (Piironen, 1985), rainbow trout (Munkittrick and Moccia, 1987), Persian sturgeon (Alavi et al., 2006), and cod (Rouxel et al., 2008). In fish, several factors have been reported that regulate the composition of seminal plasma (Ciereszko, 2008; Alavi et al., 2008b). It has already been shown in the literature that there are several correlations between seminal plasma composition and sperm motility in some species: *Salmo salar* (Hwang and Idler, 1969), *Cyprinus carpio* (Kruger et al., 1984), rainbow trout (Lahnsteiner et al., 1998), Persian sturgeon, *Acipenser persicus* (Alavi et al., 2004), and *Oncorhynchus tshawytscha* (Rosengrave et al., 2009). Lahnsteiner et al. (1996) reported a correlation between sperm motility and seminal plasma composition in bleak *Alburnus alburnus*, a cyprinid, and suggested that this correlation might indicate which components of the seminal plasma influence sperm motility. Seminal plasma pH may affect final maturation of spermatozoa. Also, duration of sperm motility in males could be influenced by the changes of semen pH (Sahinoz et al., 2008). In cyprinids, it has been shown that extracellular and intracellular pH as well as the ionic composition of the seminal plasma influence the initiation and duration of sperm motility (Marian et al., 1997). The pH of the seminal plasma of roach during breeding season ranged from 7.45 ± 0.07 to 7.95 ± 0.07. The highest sperm pH was observed in males in April (the end of reproductive season). Limit information is available on the organic composition of the carp semen. No significant changes were observed in terms of the seminal plasma organic composition in this study. Some energetic substrates such as glucose are found in the seminal plasma and the sperm but in small amounts (Kruger et al., 1984). The presence of glucose in seminal plasma was associated with high energy demand of the tests during spermatogenesis or with lipid synthesis of spermatozoa (Soengas et al., 1993). The protein content highly varied during the year (Billard and Cosson, 1990). White and Macleod (1963) indicated that protein has a protective role. During spawning period lower protein concentrations have been observed in seminal plasma of rainbow trout (Ciereszko et al., 1996). Seminal plasma lipids are associated with metabolism in spermatozoa (Piironen, 1994). While cholesterol was found in the seminal plasma of freshwater fish (Billard et al., 1995), there is not enough information about its role. Lipid and cholesterol might have a protective effect against environmental changes (especially temperature) when semen is released.

CONCLUSION

The results indicate that sperm movement duration, percentage of motile spermatozoa, Ca^{2+}, Mg^{2+}, and cholesterol were comparatively higher in March than in the other months of the spawning migration. This indicates a better quality of semen in this period. Hopefully the information

on the spermatological and biochemical characteristics of *Rutilus rutilus caspicus* presented in this study will help in optimizing the selection of high-quality male donors for aquaculture and artificial spawning performance.

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